Early Detection of Rejection after Heart Transplantation by a Universal Digital PCR Method.

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Abstract

Background:
Acute allograft rejection (AR) is a major complication after heart transplantation (HTx). Current standard for early detection of rejection is percutaneous-transvenous endomyocardial biopsy (EMB), a procedure, which is burdening, associated with risk for serious complications and false negative results, which can occur due to the patchy nature of AR. The quantification of Graft derived cell-free DNA (GcfDNA) is reported as biomarker for graft integrity, which can early detect AR in solid organ transplantation, only requiring a simple blood draw. Aim of the study was to investigate the diagnostic use of GcfDNA during the first year after HTx.

Methods:
30 Patients (23m,7f) undergoing HTx were included with an age range of 26 to 69 years, of which 26 (81%) survived the first year. 16 samples were drawn per patient during the first year post Tx. Immunosuppression was based on CNI or Tacrolimus with Mycophenolate and 6 patients received an mTOR inhibitor. EMB-proven AR occurred in 13 Patients, where two had a Grade 2 AR after more than 120 days post HTx. GcfDNA was measured with a modified published digital PCR method, based on an universal probe set. The data were calculated as percentage of graft cfDNA per total cfDNA and as absolute values in copies/ml (cp/ml) of plasma.

Results:
Immediately after engraftment, the GcfDNA was high with a median of 4.3% (IQR: 1.9-5.2) and 3.478cp/ml (IQR:1.733 - 8.172), and decreased with an approx. half-life of 3.3 days (%) and 4.5 days (cp/ml). In uncomplicated courses the levels after 2 weeks were below a threshold of 0.6% and 160cp/ml respectively (95th percentile). In the initial 2 weeks distinct increases in individual courses are better suited to indicate a rejection, since the GcfDNA and total cfDNA levels show high inter-individual variability during the post Tx decay period. In two cases of late rejections, both at one year with samples at the time of EMB-proven AR levels of 3% (5694cp/ml) and 11% GcfDNA (5512cp/ml) were detected. In three other cases of EMB-proven AR the GcfDNA increased from a) 0.15% to 2.88%, where the first significant change was seen after 6 months and the rejection was diagnosed after 12 months, b) in one earlier EMB-proven AR (2 months) GcfDNA increased beginning at 1 month to 3% and was falling to 0.6% after successful treatment. The third case had a subtle steady increase from 0.2 to 0.7% beginning 2 months before diagnosis of AR at 6 months; GcfDNA reverted to 0.2% after treatment.

Conclusion:
A modified dPCR method without pre-amplification was used to quantify GcfDNA. The measurements can be done within one working day at reasonable costs. Compared to earlier observations in liver recipients, the post repulsion phase is characterized by a longer recovery time of the graft as assessed by the GcfDNA half-life. During this phase increases of GcfDNA are subtle, but distinguishable during AR. After that period, rejections can be early distinguished by increasing and elevated GcfDNA levels, which in the later phase is characterized by early and sustained increasing levels up to 6 months before diagnosis of AR.

Transplant Liquid Biopsy Test

Healthy

1. Collect cell-free DNA from recipients plasma
2. Identify informative SNPs out of a preselected set of 38 assays
3. Quantify graft-derived cell-free DNA by droplet digital PCR (ddPCR)

Organ damage

Fractional GcfDNA (%) concentrations in early phase after engraftment in complication free patients.

Immediately after engraftment, the GcfDNA was high with a median of 4.3% (IQR: 1.9-5.2) and 3.478cp/ml (IQR:1.733 - 8.172), and decreased with an approx. half-life of 3.3 days (%) and 4.5 days (cp/ml). In uncomplicated courses the levels after 2 weeks were below a threshold of 0.6% and 160cp/ml respectively (95th percentile). These values were, therefore, defined as healthy organ thresholds.

Results – Time-Courses in Patients with Rejections

GcfDNA (%) time-course for one patient with late biopsy proven rejection (red line). Green dashed line = healthy organ threshold (0.6%).

GcfDNA (%) increased beginning at 1 month to 3% and was falling below 0.6% after successful treatment (red line). Green dashed line = healthy organ threshold (0.6%).

GcfDNA (%) time-course for one patient showing a subtle steady increase from 0.2 to 0.7% beginning 2 months before diagnosis of AR at 6 months; GcfDNA reverted to 0.2% after treatment (red line). Green dashed line = healthy organ threshold (0.6%).

Results – Post-Surgery Decline

Absolute GcfDNA (cp/ml plasma) concentrations in early phase after engraftment in complication free patients.

Absolute GcfDNA (cp/ml plasma) concentrations in patients without complications.

Results – Rejection-Free Patients

Absolute GcfDNA (cp/ml plasma) concentrations in patients without complications.

Fractional GcfDNA (%) concentrations in patients without complications.

In uncomplicated courses the levels after 2 weeks were below 0.6% and 160cp/ml respectively (95th percentile). These values were, therefore, defined as healthy organ thresholds.